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RAW SEQUENCE LISTING  
 PATENT APPLICATION: US/10/017,145

DATE: 01/10/2002  
 TIME: 15:51:00

Input Set : A:\ES.txt  
 Output Set: N:\CRF3\01102002\J017145.raw

**ENTERED**

3 <110> APPLICANT: Brookhaven Science Associates  
 4 Shanklin, John  
 6 <120> TITLE OF INVENTION: Mutant Fatty Acid Desaturase and Methods for Directed  
 Mutagenesis  
 8 <130> FILE REFERENCE: CIP of 09/328,550 filed June 9, 1999; which was a CIP of  
 09/233,856 filed  
 W--> 9 January 19, 1999  
 C--> 11 <140> CURRENT APPLICATION NUMBER: US/10/017,145  
 C--> 11 <141> CURRENT FILING DATE: 2001-12-14  
 11 <150> PRIOR APPLICATION NUMBER: 09/328,550  
 12 <151> PRIOR FILING DATE: 1999-06-09  
 14 <160> NUMBER OF SEQ ID NOS: 13  
 16 <170> SOFTWARE: PatentIn version 3.1  
 18 <210> SEQ ID NO: 1  
 19 <211> LENGTH: 363  
 20 <212> TYPE: PRT  
 21 <213> ORGANISM: Ricinus communis  
 23 <220> FEATURE:  
 24 <221> NAME/KEY: misc\_feature  
 25 <223> OTHER INFORMATION: ricinus communis delta 9 18:0 Acyl ACP Desaturase  
 28 <400> SEQUENCE: 1  
 30 Ala Ser Thr Leu Lys Ser Gly Ser Lys Glu Val Glu Asn Leu Lys Lys  
 31 1 5 10 15  
 34 Pro Phe Met Pro Pro Arg Glu Val His Val Gln Val Thr His Ser Met  
 35 20 25 30  
 38 Pro Pro Gln Lys Ile Glu Ile Phe Lys Ser Leu Asp Asn Trp Ala Glu  
 39 35 40 45  
 42 Glu Asn Ile Leu Val His Leu Lys Pro Val Glu Lys Cys Trp Gln Pro  
 43 50 55 60  
 46 Gln Asp Phe Leu Pro Asp Pro Ala Ser Asp Gly Phe Asp Glu Gln Val  
 47 65 70 75 80  
 50 Arg Glu Leu Arg Glu Arg Ala Lys Glu Ile Pro Asp Asp Tyr Phe Val  
 51 85 90 95  
 54 Val Leu Val Gly Asp Met Ile Thr Glu Glu Ala Leu Pro Thr Tyr Gln  
 55 100 105 110  
 58 Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala Ser  
 59 115 120 125  
 62 Pro Thr Ser Trp Ala Ile Trp Thr Arg Ala Trp Thr Ala Glu Glu Asn  
 63 130 135 140  
 66 Arg His Gly Asp Leu Leu Asn Lys Tyr Leu Tyr Leu Ser Gly Arg Val  
 67 145 150 155 160  
 70 Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser Gly  
 71 165 170 175  
 74 Met Asp Pro Arg Thr Glu Asn Ser Pro Tyr Leu Gly Phe Ile Tyr Thr  
 75 180 185 190  
 78 Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala Arg  
 79 195 200 205  
 82 Gln Ala Lys Glu His Gly Asp Ile Lys Leu Ala Gln Ile Cys Gly Thr

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86 Ile Ala Ala Asp Glu Lys Arg His Glu Thr Ala Tyr Thr Lys Ile Val  
 87 225 230 235 240  
 90 Glu Lys Leu Phe Glu Ile Asp Pro Asp Gly Thr Val Leu Ala Phe Ala  
 91 245 250 255  
 94 Asp Met Met Arg Lys Lys Ile Ser Met Pro Ala His Leu Met Tyr Asp  
 95 260 265 270  
 98 Gly Arg Asp Asp Asn Leu Phe Asp His Phe Ser Ala Val Ala Gln Arg  
 99 275 280 285  
 102 Leu Gly Val Tyr Thr Ala Lys Asp Tyr Ala Asp Ile Leu Glu Phe Leu  
 103 290 295 300  
 106 Val Gly Arg Trp Lys Val Asp Lys Leu Thr Gly Leu Ser Ala Glu Gly  
 107 305 310 315 320  
 110 Gln Lys Ala Gln Asp Tyr Val Cys Arg Leu Pro Pro Arg Ile Arg Arg  
 111 325 330 335  
 114 Leu Glu Glu Arg Ala Gln Gly Arg Ala Lys Glu Ala Pro Thr Met Pro  
 115 340 345 350  
 118 Phe Ser Trp Ile Phe Asp Arg Gln Val Lys Leu  
 119 355 360  
 122 <210> SEQ ID NO: 2  
 123 <211> LENGTH: 1092  
 124 <212> TYPE: DNA  
 125 <213> ORGANISM: Ricinus communis  
 127 <220> FEATURE:  
 128 <221> NAME/KEY: misc\_feature  
 129 <223> OTHER INFORMATION: residues 138 to 1239 of open reading frame  
 132 <400> SEQUENCE: 2  
 133 gcctctaccc tcaagtctgg ttctaaggaa gttgagaatc tcaagaagcc tttcatgcct 60  
 135 cctcgggagg tacatgttca gtttaccat tctatgccac cccaaaagat tgagatcttt 120  
 137 aaatccctag acaaattgggc tgaggagaac attctggttc atctgaagcc agttgagaaa 180  
 139 tggggcaac cgcaggattt tttgcagat cccgcctctg atggatttga tgagcaagtc 240  
 141 agggaaactca gggagagagc aaaggagatt cctgatgatt atttgttgtt tttgggttgg 300  
 143 gacatgataa cgaaagaagc cttcccact tatcaaaca tgctgaatac cttggatgga 360  
 145 gttcgggatg aaacaggtgc aagtctact tcttggcaa tttggacaag ggcatggact 420  
 147 gccgaagaga atagacatgg tgacctcctc aataagtatc tctacctatc tggacgagtg 480  
 149 gacatgagggc aaatttggaaa gacaattcaa tatttgattt gttcaggaat ggatccacgg 540  
 151 acagaaaaca gtccataacct tgggttcatc tatacatcat tccaggaaag ggcaaccttc 600  
 153 atttctcatg ggaacactgc ccgacaagcc aaagagcatg gagacataaa gttggctcaa 660  
 155 atatgtggta caattgctgc agatgagaag cgccatgaga cagcctacac aaagatagtg 720  
 157 gaaaaactct ttgagattga tcctgtatgaa actgtttgg ctttgtctga tatgtatgaga 780  
 159 aagaaaaattt ctatgcctgc acacttgatg tatgtatggcc gagatgataa tcttttgac 840  
 161 cactttcag ctgttgcgc gctcttgcgtt gtcacacag caaaggatta tgcagatata 900  
 163 ttggagttct tgggtggcag atggaagggtt gataaactaa cgggccttgc agctgaggga 960  
 165 caaaaaggctc aggactatgt ttgtcggtt cctccaagaa tttagaaggct ggaagagaga 1020  
 167 gctcaaggaa gggcaagga agcacccacc atgccttca gctggatttt cgataggcaa 1080  
 169 gtgaagctgt ag 1092  
 172 <210> SEQ ID NO: 3  
 173 <211> LENGTH: 34  
 174 <212> TYPE: DNA  
 C--> 175 <213> ORGANISM: Artificial

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177 <220> FEATURE:  
 178 <221> NAME/KEY: misc\_feature  
 179 <223> OTHER INFORMATION: PCR primer; sequence flanking unique XbaI site at the 5' end  
 of t

180 he open reading frame

183 &lt;400&gt; SEQUENCE: 3

184 gtgagcggat aacaatttca cacagtctag aaat

34

187 &lt;210&gt; SEQ ID NO: 4

188 &lt;211&gt; LENGTH: 72

189 &lt;212&gt; TYPE: DNA

C--&gt; 190 &lt;213&gt; ORGANISM: Artificial

192 &lt;220&gt; FEATURE:

193 &lt;221&gt; NAME/KEY: misc\_feature

194 &lt;222&gt; LOCATION: (56)..(57)

195 &lt;223&gt; OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n" indicates

196 the presence of either C, A, T or G at that nucleotide position

199 &lt;400&gt; SEQUENCE: 4

W~~C~~> 200 ccaaattgcc caagacgtcg gacttgcacc tgtttcatcc cgaactccat ccaamnnatt 60  
202 cagcattgtt tg 72

205 &lt;210&gt; SEQ ID NO: 5

206 &lt;211&gt; LENGTH: 31

207 &lt;212&gt; TYPE: DNA

C--&gt; 208 &lt;213&gt; ORGANISM: Artificial

210 &lt;220&gt; FEATURE:

211 &lt;221&gt; NAME/KEY: misc\_feature

212 &lt;223&gt; OTHER INFORMATION: PCR primer

215 &lt;400&gt; SEQUENCE: 5

216 gaaaacagggtg caagtcggac gtcttggca a 31

219 &lt;210&gt; SEQ ID NO: 6

220 &lt;211&gt; LENGTH: 26

221 &lt;212&gt; TYPE: DNA

C--&gt; 222 &lt;213&gt; ORGANISM: Artificial

224 &lt;220&gt; FEATURE:

225 &lt;221&gt; NAME/KEY: misc\_feature

226 &lt;223&gt; OTHER INFORMATION: PCR primer

229 &lt;400&gt; SEQUENCE: 6

230 gttttctgtc cgccggatcca ttctctg 26

233 &lt;210&gt; SEQ ID NO: 7

234 &lt;211&gt; LENGTH: 34

235 &lt;212&gt; TYPE: DNA

C--&gt; 236 &lt;213&gt; ORGANISM: Artificial

238 &lt;220&gt; FEATURE:

239 &lt;221&gt; NAME/KEY: misc\_feature

240 &lt;223&gt; OTHER INFORMATION: PCR primer

243 &lt;400&gt; SEQUENCE: 7

244 gtgagcggat aacaatttca cacagtctag aaat

34

247 &lt;210&gt; SEQ ID NO: 8

248 &lt;211&gt; LENGTH: 30

249 &lt;212&gt; TYPE: DNA

C--&gt; 250 &lt;213&gt; ORGANISM: Artificial

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252 <220> FEATURE:
253 <221> NAME/KEY: misc_feature
254 <223> OTHER INFORMATION: PCR primer
257 <400> SEQUENCE: 8
258 cacgaggccc tttcggttcc aagaattctc 30
261 <210> SEQ ID NO: 9
262 <211> LENGTH: 28
263 <212> TYPE: DNA
C--> 264 <213> ORGANISM: Artificial
266 <220> FEATURE:
267 <221> NAME/KEY: misc_feature
268 <223> OTHER INFORMATION: PCR primer
271 <400> SEQUENCE: 9
272 ttgataagtg ggaaggggctt cttccgtt 28
275 <210> SEQ ID NO: 10
276 <211> LENGTH: 66
277 <212> TYPE: DNA
C--> 278 <213> ORGANISM: Artificial
280 <220> FEATURE:
281 <221> NAME/KEY: misc_feature
282 <222> LOCATION: (41)..(43)
283 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n"
indicates
284      the presence of either C, A, T or G and in which "k" indicates t
285      he presence of either T or G.
288 <220> FEATURE:
289 <221> NAME/KEY: misc_feature
290 <222> LOCATION: (32)..(34)
291 <223> OTHER INFORMATION: PCR primer is degenerate oligonucleotide in which "n"
indicates th
292      e presence of either C, A T, or G at that nucleotide position and
293      in which "k" indicates either T or G
296 <220> FEATURE:
297 <221> NAME/KEY: misc_feature
298 <222> LOCATION: (44)..(46)
299 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n"
indicates
300      the presence of either C, A, T, or G at that nucleotide position
301      and in which "k" indicates the presence of either T or G.
304 <400> SEQUENCE: 10
W-4> 305 aacggaagaa gccctccca cttatcaaac annkctgaat nnknknkgatg gagttcggga 60
307 tgaaac 66
310 <210> SEQ ID NO: 11
311 <211> LENGTH: 26
312 <212> TYPE: DNA
C--> 313 <213> ORGANISM: Artificial
315 <220> FEATURE:
316 <221> NAME/KEY: misc_feature
317 <223> OTHER INFORMATION: PCR primer
320 <400> SEQUENCE: 11
321 tccattcctg aaccaatcaa atattg 26
324 <210> SEQ ID NO: 12

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**RAW SEQUENCE LISTING**

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TIME: 15:51:00

Input Set : A:\ES.txt

Output Set: N:\CRF3\01102002\J017145.raw

325 <211> LENGTH: 70  
326 <212> TYPE: DNA  
C--> 327 <213> ORGANISM: Artificial  
329 <220> FEATURE:  
330 <221> NAME/KEY: misc\_feature  
331 <222> LOCATION: (22)..(24)  
332 <223> OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n" indicates  
333 the presence of either C, A, T or G at that nucleotide position  
334 and in which "k" indicates the presence of either T or G at that  
335 nucleotide position.  
338 <220> FEATURE:  
339 <221> NAME/KEY: misc\_feature  
340 <222> LOCATION: (28)..(30)  
341 <223> OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n" indicates  
342 the presence of either C, A, T or G at that nucleotide position  
343 and in which "k" indicates the presence of either T or G at that  
344 nucleotide position.  
347 <220> FEATURE:  
348 <221> NAME/KEY: misc\_feature  
349 <222> LOCATION: (49)..(51)  
350 <223> OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n" indicates  
351 the presence of either C, A, T or G at that nucleotide position  
352 and in which "k" indicates the presence of either T or G at that  
353 nucleotide position.  
356 <400> SEQUENCE: 12  
W-OK 357 ttgattggtt caggaatgga tnnkcggnnk gaaaacagtc cataccttnn kttcatctat 60  
359 acatcattcc 70  
362 <210> SEQ ID NO: 13  
363 <211> LENGTH: 30  
364 <212> TYPE: DNA  
C--> 365 <213> ORGANISM: Artificial  
367 <220> FEATURE:  
368 <221> NAME/KEY: misc\_feature  
369 <223> OTHER INFORMATION: PCR primer  
372 <400> SEQUENCE: 13  
373 qcaaaaqcca aaacqqtacc atcaqqatca 30

## VERIFICATION SUMMARY

PATENT APPLICATION: US/10/017,145

DATE: 01/10/2002

TIME: 15:51:01

Input Set : A:\ES.txt

Output Set: N:\CRF3\01102002\J017145.raw

L:9 M:259 W: Allowed number of lines exceeded, <130> FILE REFERENCE:  
L:11 M:270 C: Current Application Number differs, Replaced Current Application No  
L:11 M:271 C: Current Filing Date differs, Replaced Current Filing Date  
L:175 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:3  
L:190 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:4  
L:200 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:4  
L:208 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:5  
L:222 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:6  
L:236 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:7  
L:250 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:8  
L:264 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:9  
L:278 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:10  
L:305 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:10  
L:313 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:11  
L:327 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:12  
L:357 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:12  
L:365 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:13